



# Bone calcium turnover during pregnancy and lactation in women with low calcium diets is associated with calcium intake and circulating insulin-like growth factor 1 concentrations<sup>1-3</sup>

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## ABSTRACT

**Background:** Few data exist on longitudinal changes in bone calcium turnover rates across pregnancy and lactation.

**Objective:** Our aim was to characterize calcium kinetic variables and predictors of these changes across pregnancy and early lactation in women with low calcium intakes.

**Design:** Stable calcium isotopes were administered to 10 Brazilian women during early pregnancy (EP; weeks 10–12 of gestation), late pregnancy (LP; weeks 34–36 of gestation), and early lactation (EL; 7–8 wk postpartum). Multicompartmental modeling was used to assess the rates of bone calcium turnover in relation to calcium intakes and circulating concentrations of parathyroid hormone (PTH), insulin-like growth factor 1, and 1,25-dihydroxyvitamin D.

**Results:** Rates of bone calcium deposition increased significantly from EP to LP ( $P = 0.001$ ) and were significantly associated with serum PTH during LP ( $P \leq 0.01$ ). Rates of bone calcium resorption were also higher during LP and EL than during EP ( $P \leq 0.01$ ) and were associated with both PTH ( $P \leq 0.01$ ) and IGF-1 ( $P \leq 0.05$ ) during LP but not during EL. Net balance in bone calcium turnover was positively associated with dietary calcium during EP ( $P \leq 0.01$ ), LP ( $P \leq 0.01$ ), and EL ( $P \leq 0.01$ ). The mean ( $\pm$ SD) calcium intake was  $463 \pm 182$  mg/d and, in combination with insulin-like growth factor 1, explained 68–94% of the variability in net bone calcium balance during pregnancy and lactation.

**Conclusions:** Net deficits in bone calcium balance occurred during pregnancy and lactation. Increased dietary calcium intake was associated with improved calcium balance; therefore, greater calcium intakes may minimize bone loss across pregnancy and lactation in women with habitual intakes of  $<500$  mg calcium/d. *Am J Clin Nutr* 2006;83:317–23.

**KEY WORDS** Pregnancy, stable isotopes, calcitropic hormones, insulin-like growth factor 1, bone mass

## INTRODUCTION

Pregnancy puts a significant stress on maternal calcium economy because of the 30 g calcium that is transferred to the fetus over the 280-d gestation period (1). This demand is highest during the final trimester of pregnancy, when approximately two-thirds of the total calcium requirement of the fetus is transferred across the placenta (1). A similarly high calcium demand is present throughout the course of lactation to accommodate the  $\approx 300$  mg calcium that is required each day to meet the demands

of breast-milk production in exclusively breastfeeding women (2). Many questions concerning the metabolic changes in calcium dynamics and bone turnover that occur to accommodate this calcium demand in pregnant and lactating women remain, and few data are available in women with habitually low calcium intakes.

The ability of pregnant and lactating women with low dietary calcium intakes to modify calcium partitioning and bone calcium turnover is receiving increased attention with respect to its effects on both maternal and neonatal bone health. Low calcium intake during pregnancy has been associated with reduced bone mineral content in newborns (3), and insufficient maternal calcium intake during pregnancy may adversely affect fetal femur length and maternal bone mass in pregnant adolescents (4, 5).

Stable isotopic studies allow for the safe characterization of longitudinal changes in calcium absorption and rates of bone turnover during pregnancy and into the lactation period. Nearly all longitudinal stable calcium isotope studies published to date across both pregnancy and lactation have measured only calcium absorption and have not examined postdosing isotopic clearance in serum and urine to assess the rates of bone calcium turnover (6–9). At present, only 2 calcium kinetic studies conducted in pregnant or lactating women have been published (10, 11). Of these, only one studied a subset of women across both physiologic states (11). Additional limitations of the calcium kinetic data published to date are that the populations studied consumed  $\approx 800$  mg calcium/d (11) or that the studies were conducted in

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lactating women who received acute low (370 mg/d) or high (1500 mg/d) calcium diets (10). Although stable isotope studies of calcium absorption have been published in lactating Gambian women with habitually low calcium intakes that averaged 283 mg/d (12), the degree to which rates of bone calcium deposition and resorption can be altered in response to pregnancy and lactation in women with habitually low calcium intakes is not known.

Recently, we reported that calcium absorption during pregnancy and lactation was substantially higher in a cohort of Brazilian women with habitually low intakes of calcium ( $\approx 500$  mg/d) than the absorption that is typically reported in pregnant and lactating women (8). The purpose of the present study was to additionally assess the magnitude and predictors of longitudinal changes in both the rates of bone calcium deposition and resorption and the net balance in bone calcium turnover in response to the calcium demands of pregnancy and lactation in the same group of Brazilian women.

## SUBJECTS AND METHODS

### Subject characteristics

Subjects were recruited from the Maternidade Escola of the Federal University of Rio de Janeiro (UFRJ), Brazil, during their first prenatal care visit, which occurred, on average, at 8 wk gestation. At this time, each woman was given a full explanation of all study procedures. Ten women of low socioeconomic status volunteered to participate in the study. The women were healthy, multigravidae, and were of mixed European and African ancestry. The women maintained their habitual dietary habits during the study. All women received standard prenatal care throughout pregnancy and were provided with prenatal iron supplements (as ferrous sulfate) containing no calcium. None of the study participants consumed calcium supplements. Detailed characteristics of this study population have been previously described (8). The study was approved by the Committee for the Protection of Human Subjects at the University of California, Berkeley, and by the Ethical Committee of Maternidade Escola, Universidade Federal do Rio de Janeiro, Brazil. Informed written consent was obtained from all subjects.

### Study design

Calcium kinetic studies were undertaken at 3 time points in each woman: weeks 10–12 of gestation (early pregnancy, EP), weeks 34–36 of gestation (late pregnancy, LP), and 7–8 wk postpartum (early lactation, EL). All women exclusively breastfed their infants during the third calcium kinetic study (EL). At each time point, the following were assessed: habitual dietary calcium intake, 24-h urinary calcium excretion, serum hormone and cytokine concentrations, fractional intestinal calcium absorption, and calcium kinetics. Nine women were studied at the 3 time points. One woman was studied at EP and at EL, but not at LP because she delivered prematurely. The calcium absorption, urinary calcium excretion, and serum hormone concentrations of the 9 women who were studied longitudinally during pregnancy and early lactation were previously reported (8).

### Procedures

Dietary intake was assessed from weighed food intake records kept by the subjects for 3 consecutive days before each of the 3

calcium kinetic studies, as previously described (8). The nutrient content of the diet was determined by using the Food Processor nutrient database (ESHA Research, Salem, OR) that was adapted for Brazilian foods with the use of published food-composition data (13).

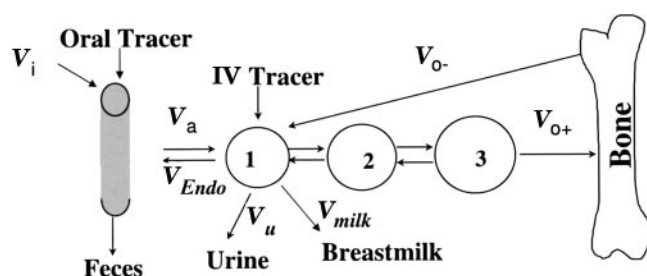
After an overnight fast, stable calcium isotopes were administered to the subjects at the Maternidade Escola-UFRJ with a double-tracer isotope technique in which  $^{42}\text{Ca}$  was administered intravenously and  $^{46}\text{Ca}$  was administered orally. Isotopic solutions were prepared for oral and intravenous dosing from enriched calcium carbonate ( $^{42}\text{CaCO}_3$ , 93.58% enrichment, and  $^{46}\text{CaCO}_3$ , 6.1% enrichment; Trace Sciences International, Toronto, Canada).

On the morning of each kinetic study, the weights and heights of the subjects were recorded and an indwelling catheter was placed in the antecubital vein from which a baseline (ie, fasting) blood sample (10 mL) and all other blood samples were drawn with the use of Monovette syringes (Sarstedt, Hayward, CA) that contained heparin-coated beads. The subjects received a standard breakfast containing  $\approx 74$  mg calcium, followed 10–15 min later by ingestion of  $10\ \mu\text{g}\ ^{46}\text{Ca}$  in 50 mL water. Immediately after the oral dosing,  $5.0\ \text{mg}\ ^{42}\text{Ca}$  was administered intravenously over 1–2 min with a butterfly infusion set into the antecubital vein of the arm opposite that used for sampling. The exact amount of the isotope solution infused was determined by weighing the syringe before and after the infusion. Blood samples (8 mL) were taken via the catheter after the  $^{42}\text{Ca}$  infusion at the following time points: 4, 8, 12, 16, 20, 30, 45, and 60 min, and 2, 3, 6, 9, 12, and 24 h. A complete 24-h urine collection was obtained during the test day. Total urine weight was measured to the nearest 0.1 g. Spot urine samples, which were collected in the early morning, midafternoon, and late evening, were obtained on days 2–5 after isotope administration. Baseline samples were collected before the start of each subsequent isotopic study to validate that the enrichment had returned to baseline levels before each subsequent study.

Urine samples were acidified (final pH: 2) by adding concentrated trace metal-grade hydrochloride (Fisher Scientific, Fairlawn, NJ), and aliquots were stored at  $-20\ ^\circ\text{C}$  until analyzed. Blood samples were kept refrigerated ( $4\ ^\circ\text{C}$ ) and processed  $\leq 2$  h after being drawn. Aliquots of plasma were kept at  $-20\ ^\circ\text{C}$  until analyzed.

Calcium concentration in plasma and urine samples was measured by atomic absorption spectrophotometry (Perkin Elmer AA3300, Boston, MA) in samples appropriately diluted with 0.5% lanthanum chloride (Sigma, St Louis, MO) in 0.5 mol HCl/L (Optima; Fisher Scientific). Calcium was extracted from plasma and urine samples with a calcium oxalate precipitation method (14). Calcium isotopic ratios in processed samples were measured with magnetic sector thermal-ionization mass spectrometry (Thermoquest; Triton TI, Bremen, Germany). The ratio of each administered tracer to  $^{43}\text{Ca}$  was measured, and the degree to which this ratio was increased over the natural abundance ratio was calculated. Relative SDs typically achieved for the isotopic ratios measured were  $<0.05\%$  for  $^{42}\text{Ca}:^{43}\text{Ca}$  and  $<0.1\%$  for  $^{46}\text{Ca}:^{43}\text{Ca}$ . The percentage absorption of calcium was calculated as the relative recovery of the oral and the intravenous tracer in the 24-h urine that was collected after the isotope administration (15).

Calcium kinetic measures of bone calcium deposition ( $\text{Vo}+$ ) were measured by tracing the rate of disappearance of  $^{42}\text{Ca}$  from



**FIGURE 1.** Calcium kinetic model. To assess calcium kinetics and absorption, one stable calcium isotope ( $^{46}\text{Ca}$ ) is administered orally and is absorbed across the gastrointestinal tract into the central exchangeable calcium pool (compartment 1). A second stable calcium isotope ( $^{42}\text{Ca}$ ) was injected directly into compartment 1. Clearance of the intravenous (IV) tracer in serum and urine over the 120-h postdosing interval was used to assess rates of bone calcium deposition ( $V_{o+}$ ) and resorption ( $V_{o-}$ ) after control for urinary ( $V_a$ ) and endogenous ( $V_{\text{Endo}}$ ) fecal calcium losses. Endogenous fecal calcium losses were set at  $1.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  on the basis of previous literature values (20). Compartments 2 and 3 are time intermediaries in bone matrix formation. During pregnancy,  $V_{o+}$  reflects the loss of calcium into both maternal and fetal bone. The partitioning of these losses can be estimated by using rates of fetal calcium deposition typically reported during the third trimester of pregnancy (18, 19). During lactation, an additional calcium loss from compartment 1 is added into the model to account for calcium loss into breastmilk ( $V_{\text{milk}}$ ). The losses into breastmilk were estimated at 200 mg/d according to previous data in Brazilian women (21). The net difference between  $V_{o+}$  and  $V_{o-}$  is a measure of net bone calcium balance.  $V_a$ , rate of calcium absorption;  $V_i$ , dietary calcium intake.

plasma and spot urine samples over the 5-d postdosing collection period. A multicompartimental model, based on the model proposed by Neer et al (16) and SAAM (Simulation, Analysis, and Modeling) software, was used for these determinations as detailed previously (17). In this model,  $V_{o+}$  and the rate of bone calcium resorption ( $V_{o-}$ ) were measured by tracing the disappearance of the intravenous tracer for the 120-h postdosing period and after accounting for fractional calcium absorption, losses from the system in urine, and endogenous fecal secretion ( $V_{\text{Endo}}$ ) (Figure 1). During pregnancy,  $V_{o+}$  also includes the loss of calcium into fetal bone; however, these 2 rates cannot be distinguished from one another. To estimate maternal compared with fetal bone calcium deposition, calculations were undertaken to partition this loss with the use of the estimated rate of fetal bone calcium deposition of 350 mg/d, which has a basis on reported data from similar stages of gestation (18, 19). During the LP study, net maternal calcium retention is reported both with and without this estimate. Rates of calcium losses from the system in urine were calculated in each study from the 24-h urine collection, and  $V_{\text{Endo}}$  was estimated at  $1.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ . Previous studies in women have found that  $V_{\text{Endo}}$  is only marginally affected by calcium intake and is not significantly changed during pregnancy (11, 20). During the lactation period, calcium losses in breast milk were also included in the model and were estimated to average 200 mg/d on the basis of previous data obtained in lactating Brazilian women at similar stages of lactation (21). Net bone calcium balance was defined as the difference between  $V_{o+}$  and  $V_{o-}$ . Negative values are indicative of a net loss of calcium from bone; positive values are indicative of a net gain in bone calcium.

Statistical analyses were performed with STATGRAPHICS (version 7; Manugistics, Cambridge, MA). Comparisons between time points were made by repeated-measures analysis of variance; given overall differences, we tested specific contrasts

**TABLE 1**  
Characteristics of the study subjects<sup>1</sup>

	Value
Age (y)	30 (21–34)
BMI before pregnancy ( $\text{kg}/\text{m}^2$ )	23 (18–27)
Parity (no.)	2.6 (2–5)
Blood hemoglobin, 10–12 wk of pregnancy (g/L)	120 (103–137)
Length of gestation (wk)	38 (33–41)
Weight gain during gestation (kg)	11.4 (6.5–18.9)
Infant birth weight (kg)	3.1 (2.1–4.4)
Infant birth length (cm)	50 (45–54)

<sup>1</sup> All values are  $\bar{x}$ ; range in parentheses.  $n = 10$ .

(EP compared with LP, LP compared with EL, and EP compared with EL). In general, we considered  $P < 0.05$  as significant, and for the contrasts we applied the Bonferroni-Dunn correction factor. Average group values at LP were used for the subject who was missing this study point. We then explored predictors of the outcomes at each time point by first examining bivariate associations. Full models, which included all variables that had shown an association with a  $P$  value  $\leq 0.10$ , were then fit. These models were then reduced in a systematic fashion to remove those variables that contributed least to each model. This was continued until we arrived at parsimonious models with variables that were statistically significant at  $P < 0.05$ .

## RESULTS

The characteristics of the study participants are presented in Table 1. The women were relatively well nourished at the time of entry into prenatal care and gained an average of 11.4 kg over pregnancy. Most women (90%) were not anemic when this measure was assessed at week 10–12 of pregnancy. The infants' average birth weights were consistent with those reported in Brazilian infants (22). Of the 10 women studied, 1 delivered prematurely (week 33 of gestation). On the basis of oral-glucose-tolerance tests that were administered at week 28 of pregnancy, none of the women had evidence of gestational diabetes.

Dietary intake of calcium did not vary significantly across pregnancy or the EL period and averaged 463 mg/d (range: 290–740 mg/d), irrespective of physiologic state (Table 2). Dairy products contributed  $<50\%$  of the daily calcium intake as previously reported (8). Although dairy intake was limited in these women, the UV-B wavelength needed to synthesize vitamin D is present throughout the year at this latitude ( $23^\circ\text{S}$  latitude). However, because we did not measure 25-hydroxyvitamin D in these women, we are unable to evaluate the effect of vitamin D status on study outcomes.

Calcium kinetic variables and biochemical indexes at each sampling point are also presented in Table 2. Rates of bone turnover ( $V_{o-}$  and  $V_{o+}$ ) increased significantly across pregnancy and remained high at EL ( $P < 0.01$ ). Net bone calcium balance increased significantly from EP to LP ( $P = 0.001$ ) but did not statistically differ from EP to EL. However, when net calcium balance at LP was corrected for estimates of fetal bone calcium deposition, the net calcium balance decreased significantly from EP to LP ( $P = 0.02$ ) but did not statistically differ between LP and EL and averaged  $-164 \text{ mg}/\text{d}$  during LP and EL.

Significant predictors of calcium kinetic parameters were explored by simple regression (Table 3). During EP,  $V_{o-}$  was



**TABLE 2**

Calcium kinetic and hormonal data during pregnancy and lactation in the study subjects<sup>1</sup>

	EP	LP	EL
Calcium intake (mg/d)	446 ± 149	514 ± 221	433 ± 180
Fractional calcium absorption	0.71 ± 0.16 <sup>a</sup>	0.88 ± 0.13 <sup>b</sup>	0.68 ± 0.19 <sup>a</sup>
True calcium absorption (mg/d)	308 ± 94 <sup>a</sup>	448 ± 172 <sup>b</sup>	284 ± 121 <sup>a</sup>
Urinary calcium (mg/d)	296 ± 137 <sup>c</sup>	216 ± 125 <sup>b</sup>	94 ± 44 <sup>a</sup>
Vo+ (mg/d)	460 ± 171 <sup>a</sup>	1015 ± 305 <sup>c</sup>	737 ± 139 <sup>b</sup>
Vo- (mg/d)	537 ± 176 <sup>a</sup>	889 ± 362 <sup>b</sup>	840 ± 147 <sup>b</sup>
Vo-:Vo+	1.338 ± 0.579 <sup>b</sup>	0.864 ± 0.155 <sup>a</sup>	1.154 ± 0.185 <sup>b</sup>
Net balance in bone calcium turnover (mg/d) <sup>2</sup>	-77 ± 149 <sup>a</sup>	126 ± 152 <sup>b</sup>	-104 ± 128 <sup>a</sup>
Net balance (mg/d) accounting for estimated fetal calcium accretion (350 mg/d)	-77 ± 149 <sup>a</sup>	-224 ± 152 <sup>b</sup>	-104 ± 128 <sup>a,b</sup>
1,25(OH) <sub>2</sub> D (ng/L)	74.6 ± 14.7 <sup>b</sup>	93.8 ± 20.2 <sup>c</sup>	56.0 ± 18.5 <sup>a</sup>
Parathyroid hormone (pmol/L)	1.21 ± 0.57 <sup>a</sup>	1.31 ± 0.56 <sup>a,b</sup>	1.71 ± 0.62 <sup>b</sup>
IGF-1 (mg/L)	208 ± 47 <sup>a</sup>	278 ± 55 <sup>b</sup>	180 ± 53 <sup>a</sup>

<sup>1</sup> All values are  $\bar{x} \pm \text{SD}$ ;  $n = 10$  ( $n = 9$  for LP). Vo-, bone calcium resorption rate; Vo+, bone calcium accretion rate; EP, early pregnancy (10–12 wk); LP, late pregnancy (34–36 wk); EL, early lactation (7–8 wk postpartum); 1,25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D; PTH, parathyroid hormone; IGF-1, insulin-like growth factor 1. True calcium absorption was calculated as the product of calcium intake and fractional calcium absorption. Values in a row with different superscript letters are significantly different,  $P < 0.05$  (ANOVA with Bonferroni-Dunn multiple comparisons test).

<sup>2</sup> Negative values are indicative of a net loss of calcium from bone; positive values are indicative of a net gain in bone calcium balance.

significantly associated with serum insulin-like growth factor 1 (IGF-1;  $P = 0.044$ ). The ratio of Vo- to Vo+ was also significantly positively associated with 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D;  $P = 0.037$ ], whereas the net balance in bone calcium was inversely associated with 1,25(OH)<sub>2</sub>D ( $P = 0.033$ ) and positively associated with parathyroid hormone (PTH;  $P = 0.020$ ). In the LP period, IGF-1 remained a significant predictor of Vo- ( $P = 0.033$ ) and also became significantly negatively associated with the net balance in bone calcium ( $P = 0.038$ ). By LP, significant associations were also evident between serum PTH concentrations and both Vo- ( $P = 0.008$ ) and Vo+ ( $P = 0.005$ ). These relations did not persist into the EL period.

As previously reported, the percentage calcium absorption was significantly positively associated with both 1,25(OH)<sub>2</sub>D ( $r = 0.80$ ,  $P < 0.05$ ) and PTH ( $r = 0.70$ ,  $P < 0.05$ ) during the EL study, but not during either the EP or LP studies (8). Similarly, serum IGF-1 was significantly inversely associated with the percentage calcium absorption during EL but not during either EP or LP (8). Relations between 1,25(OH)<sub>2</sub>D and calcium intake approached significance during the EP ( $r = -0.54$ ,  $P = 0.11$ ) and EL ( $r = -0.59$ ,  $P = 0.07$ ) periods.

The net bone calcium balance was significantly positively associated with dietary calcium intake during EP ( $P = 0.01$ ), LP ( $P = 0.009$ ), and EL ( $P < 0.001$ ). Moreover, the changes in net bone calcium balance from EP to LP and from EP to EL were positively associated with the corresponding changes in calcium

**TABLE 3**

Correlation coefficients between calcium kinetic variables and hormone concentrations and calcium intake during pregnancy and lactation<sup>1</sup>

Related variables	1,25(OH) <sub>2</sub> D	PTH	IGF-1	Calcium intake
Vo-				
EP	NS	NS	0.644 <sup>2</sup>	NS
LP	NS	0.809 <sup>3</sup>	0.706 <sup>2</sup>	NS
EL	NS	NS	NS	NS
Vo+				
EP	NS	NS	NS	NS
LP	NS	0.831 <sup>3</sup>	NS	NS
EL	NS	NS	NS	NS
Vo-:Vo+				
EP	0.663 <sup>2</sup>	NS	NS	NS
LP	NS	NS	0.679 <sup>2</sup>	-0.841 <sup>3</sup>
EL	NS	NS	NS	-0.886 <sup>4</sup>
Net balance in bone calcium <sup>5</sup>				
EP	-0.672 <sup>2</sup>	0.713 <sup>2</sup>	NS	0.761 <sup>3</sup>
LP	NS	NS	-0.693 <sup>2</sup>	0.804 <sup>3</sup>
EL	NS	0.623 <sup>2</sup>	NS	0.897 <sup>4</sup>

<sup>1</sup> 1,25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D; PTH, parathyroid hormone; IGF-1, insulin-like growth factor 1; Vo-, bone calcium resorption rate; Vo+, bone calcium accretion rate; Vo-:Vo+, the ratio of Vo- to Vo+; EP, early pregnancy (10–12 wk); LP, late pregnancy (34–36 wk); EL, early lactation (7–8 wk postpartum).

<sup>2–4</sup> There was a significant association between the outcome variable and the particular hormone or marker (simple regression): <sup>2</sup> $P < 0.05$ , <sup>3</sup> $P < 0.01$ , <sup>4</sup> $P < 0.001$ .

<sup>5</sup> Accounted for fetal calcium accretion at LP.

intake ( $r = 0.931$ ,  $P < 0.001$  and  $r = 0.847$ ,  $P < 0.01$ , respectively). In addition, the ratio of Vo- to Vo+ was significantly associated with dietary calcium intakes during both LP ( $P = 0.047$ ) and EL ( $P < 0.001$ ) (Table 3).

Significant predictors of calcium kinetic parameters were also explored by using multiple regression models that related the dependent variables to hormones and calcium intake (Table 4). During LP, PTH and calcium intake explained 74% of the variability in Vo-; PTH and 1,25(OH)<sub>2</sub>D explained 84% of the variability in Vo+; and IGF-1, 1,25(OH)<sub>2</sub>D, and calcium intake together explained 88% of the variability in net bone calcium balance. IGF-1 and dietary calcium explained 68% and 94% of the variability in the net bone calcium balance during EP and EL, respectively.

## DISCUSSION

To our knowledge, our study is the first longitudinal study of calcium kinetics in a population of pregnant and lactating women with habitually average calcium intakes of  $\approx 500$  mg/d. In these women, significant changes in PTH, IGF-1, and 1,25(OH)<sub>2</sub>D concentrations occurred across pregnancy and lactation. Despite the observed hormonal changes, which were similar to those expected during pregnancy and lactation (2, 6–11), a net positive bone calcium balance was only evident during LP, with net calcium losses of  $\approx 90$  mg/d occurring during the EP and EL period.

The first measure of calcium dynamics in the present study population was obtained during the first trimester at weeks 10–12 of gestation. At this stage of pregnancy, serum IGF-1 concentrations were positively associated with Vo-. We are

TABLE 4

Multiple regression equations relating calcium kinetic variables to hormone concentrations and calcium intake during pregnancy and lactation<sup>1</sup>

Time of study and dependent variable	Independent variable	Partial correlation coefficient	Intercept	Coefficient (full regression)	<i>P</i>	Adjusted <i>R</i> <sup>2</sup> (full regression)	<i>P</i> (full regression)
EP							
Net bone calcium balance			−151.8			0.676	0.008
	IGF-1	−0.633		−1.291	0.067		
	Calcium intake	0.838		0.771	0.005		
LP							
Vo−			575.0			0.743	0.007
	PTH	0.865		491.8	0.006		
	Calcium intake	−0.665		−0.644	0.072		
Vo+			1004			0.837	0.002
	PTH	0.928		479.0	< 0.001		
	1,25(OH) <sub>2</sub> D	−0.777		−6.571	0.023		
Net bone calcium balance			85.93			0.880	0.003
	IGF-1	−0.812		−1.140	0.027		
	1,25(OH) <sub>2</sub> D	−0.768		−2.493	0.044		
	Calcium intake	0.915		0.467	0.004		
EL							
Net bone calcium balance	IGF-1	−0.875	−200.9	−0.947	0.002	0.941	< 0.001
	Calcium intake	0.971		0.619	< 0.001		

<sup>1</sup> EP, early pregnancy (10–12 wk); LP, late pregnancy (34–36 wk); EL, early lactation (7–8 wk postpartum); Vo-, bone calcium resorption rate; Vo+, bone calcium accretion rate; PTH, parathyroid hormone; IGF-1, insulin growth factor-1; 1,25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D.

aware of no other data on the relations between IGF-1 and kinetically derived measures of bone turnover during pregnancy. However, calcium kinetic studies in nonpregnant adolescent girls also reported positive associations between IGF-1 and bone deposition (23). Moreover, no significant relation was found between IGF-1 and a marker of bone formation (osteocalcin) during the first trimester in normotensive pregnant women; however, possible relations between IGF-1 and markers of bone resorption were not addressed (24). We found no significant associations between PTH and 1,25(OH)<sub>2</sub>D concentrations and Vo- or with Vo+ during the EP period. However, the ratio of Vo- to Vo+, which indicates an increased rate of bone calcium resorption relative to formation, was significantly positively associated with 1,25(OH)<sub>2</sub>D during EP. These data may suggest that persons with the greatest calcium demand at this stage of pregnancy, as evidenced by increased 1,25(OH)<sub>2</sub>D concentrations, were also relying on increased bone calcium resorption to meet calcium demands.

The mean observed deficit in bone calcium turnover during EP was -77 mg/d in these women. This deficit was likely exacerbated by the increased urinary calcium losses that typically occur during pregnancy, which averaged ≈300 mg/d during EP in these women (8). Urinary losses decreased from EP to LP and were lower by LP than those typically measured in women who consume higher amounts of calcium; this conservation may have improved calcium balance in the women with low calcium intakes (8). It is unlikely that fetal calcium demands contributed substantially to the imbalance in bone turnover in EP, because the mean daily transfer of calcium to the fetus during the first trimester is only 2–3 mg/d (25).

Maximal fetal calcium accretion occurs during the third trimester of pregnancy, at which time fetal calcium deposition can approximate 350 mg/d (19). To accommodate this increased fetal calcium demand, significant increases in the rates of bone calcium deposition and resorption were evident in LP relative to

those observed in either the EP or EL periods. Moreover, the average ratio of Vo- to Vo+ was significantly lower during LP than in either the EP or EL periods. In addition to the observed changes in bone turnover, serum IGF-1 concentrations were also significantly increased by nearly 34% compared with those observed in EP. An increase in IGF-1 concentrations across pregnancy has been reported in other longitudinal studies of pregnancy (26–28). The increase we observed in our study cohort is comparable to the 43% increase in IGF-1 observed by Sowers et al (24) across pregnancy but is lower than the 68% increase reported by Lof et al (28) between weeks 14 and 32 of gestation.

Vo- was significantly associated with serum IGF-1 concentrations during EP and LP, and net balance in bone calcium was significantly inversely associated with serum IGF-1 during LP. These findings probably reflect the stimulatory effect of IGF-1 on bone turnover during pregnancy (29), which was apparently not compensated for by other homeostatic adjustments, such as increased intestinal calcium absorption, during LP. Our results are consistent with previous relations that were identified in a longitudinal study conducted in 16 women in whom changes in serum IGF-1 from baseline to 36 wk of gestation were significantly associated with percentage changes in several markers of bone formation and resorption (26). Serum PTH concentrations were also significantly related to measured rates of bone calcium turnover during LP in our study population. This result is consistent with our earlier finding of a significant relation between the relative increases in PTH and a biochemical measure of bone resorption (urinary N-telopeptide) between the EP and LP studies (8). In contrast, serum PTH was positively related to net balance in bone calcium during EP and EL, a finding that appears to be consistent with the anabolic properties of PTH described in skeletal tissue and in vitro studies (30).

During LP, a net positive calcium balance in bone turnover was maintained even with low calcium intakes. This balance was facilitated by a significant reduction in urinary calcium excretion




and a significant increase in fractional intestinal calcium absorption during LP compared with the values measured in EP ( $0.88 \pm 0.13$  compared with  $0.71 \pm 0.16$ , respectively;  $P < 0.05$ ). A limitation of the multicompartmental model of calcium kinetics during pregnancy is that the relative partitioning of calcium deposition into maternal compared with fetal bone cannot be assessed. However, if fetal calcium accretion is estimated by using published values that were obtained at similar stages of pregnancy (350 mg/d) (19), the average net balance in maternal bone calcium turnover in LP would decrease, rather than increase, significantly from that observed in EP, although it would still not differ significantly from EL. Unfortunately, we obtained no data on total body bone mineral content of the neonate at delivery with which to examine the possible relations between maternal calcium kinetic data and the neonatal calcium endowment at birth.

Losses of calcium in breast milk and endogenous fecal calcium losses were estimated in the mathematical modeling of these data. Breast-milk calcium losses were set at 200 mg/d according to values reported in similar cohorts of Brazilian women (21). Because the preponderance of data does not support a conservation of breast-milk calcium losses in women with habitually low calcium intakes (31), it is unlikely that these losses would be markedly lower in this group. An additional limitation of the mathematical modeling of these data relates to our estimation of endogenous fecal calcium losses. At present, no data support a reduction of these losses during pregnancy or lactation; thus, we used the accepted value of  $1.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  (20). To maintain net positive calcium retention solely by conservation of endogenous fecal calcium losses, these losses would have to be virtually eliminated during the EP and EL periods. Despite the fact that complete 5–10 d fecal collections add challenges to the implementation of these studies in settings where personnel and storage resources are often limited, additional research is warranted to directly measure these losses across pregnancy and lactation in women with low calcium intakes.

Note that bone calcium balance was significantly associated with calcium intake during early and late pregnancy. Moreover, calcium intake, in combination with circulating IGF-1 concentrations, was a significant predictor of bone calcium balance during LP and EL. This suggests that higher calcium intakes during pregnancy and early lactation may minimize maternal bone loss in women who consume  $<500 \text{ mg}$  calcium/d. Because no bone density measures were obtained, we were unable to evaluate the degree to which the observed imbalance in rates of bone calcium turnover during pregnancy and lactation may be associated with measurable changes in maternal bone mass, an issue that remains controversial. From a review of existing studies, several, but not all, longitudinal studies have reported a loss in maternal bone density during pregnancy and lactation (25). The imbalance in bone calcium turnover that we observed during EP differs from the net positive calcium balance reported in a combined balance and isotopic study by Heaney et al (11) conducted in women (during weeks 20–24 of gestation) who consumed  $\approx 800 \text{ mg}$  calcium/d during EP. Heaney hypothesized that this increased retention in the women, who had higher calcium intakes than those of our women, may augment maternal calcium reserves in anticipation of subsequent fetal demands.

In conclusion, significant changes in the rates of bone turnover occurred across pregnancy and lactation in women with habitually low calcium intakes. Despite significant hormonal changes,

the physiologic adaptations were not sufficient to prevent a negative balance in bone calcium turnover during EP. Because the net balance in bone calcium turnover was significantly related to calcium intake during pregnancy and lactation, increased calcium intake may minimize bone calcium losses across pregnancy in women with habitually low calcium intakes. Additional studies are needed to address the effects of higher calcium intakes or calcium supplementation on calcium kinetics, maternal and fetal bone mass, and longitudinal changes in bone mass and calcium kinetics across the reproductive and postweaning periods in similar cohorts of women with habitually low calcium intakes. 

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KOO was responsible for the manuscript preparation, isotopic analyses, and mathematical modeling of the data. CMD was responsible for the overall supervision of the study, was involved with the study design and implementation and the laboratory and statistical analyses, and assisted with the manuscript preparation. CLVZ was responsible for the clinical study, data collection, and statistical analyses and assisted with the manuscript preparation. SAA was responsible for the isotopic analysis of the calcium absorption data and assisted in data interpretation. EMS was responsible for the IGF-1 analyses. JCK was responsible for the conception and funding of the study and assisted with all aspects of the study implementation and analysis and with the manuscript preparation. None of the study authors had any personal or financial affiliations with the supporter of this research.

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